Runnig Title: Syllidae-Protozoan epibiosis

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The tag-along friendship: epibiotic protozoans and syllid polychaetes. Implications for the taxonomy of Syllidae (Annelida), and description of three new species of *Rhabdostyla* and *Cothurnia* (Ciliophora, Peritrichia).

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Abstract

The presence of peritrich ciliate epibionts *Rhabdostyla* sp. and *Cothurnia* sp. on Syllidae polychaetes is reported here for the first time, including the description of three new species of epibiont ciliates. The protozoans were mainly found at intersegmental furrows, close to parapodial bases of newly collected specimens of *Syllis magdalena*, *Syllis* sp., *Salvatoria* sp. and *Salvatoria concinna* n. comb. from Chile, *Syllis prolifera* from Spain and *Prosphaerosyllis magnoculata* from New Zealand. In addition, epibiont protozoans were found on the dorsal surface, nuchal organs, mouth opening and anterior cirri of *Syllis elongata* from Peru, and the ventral surface of *Typosyllis macropectinans* from Australia. The discovery of protozoan epibionts on syllid polychaetes has important taxonomic implications that are discussed here. The presence of papillae that

are in fact misinterpreted ciliate epibionts has been considered a valid character to distinguish among species or to erect new species of Syllidae, and thus the real origin of papillae-like structures in polychaetes, should be carefully assessed.

Key words: Syllidae-protozoan epibiosis, symbiosis, peritrich ciliates, new species, taxonomy.

Introduction

Polychaetes are known for their facility to establish a wide array of interconnections with other organisms, including different symbiotic associations (Martin & Britayev, 1998). Symbiosis in a wider sense includes any association between two organisms living together with a gradient of positive or negative consequences for at least one of them (Henry, 1966; Boucher, James & Keeler, 1982; Bush *et al.*, 2001) which, in some cases, leads some or all partners to lose the ability to live as free-living organisms (Rhode, 1981). Such associations are named differently depending on the degree of “intimacy” and the consequences for the involved partners, and include mutualism, commensalism and parasitism, the latter two widely reported in polychaetes (Martin & Britayev, 1998).

Additionally, depending on the location of the symbiont relative to the host, the association can be considered endobiotic, when the symbiont lives inside the host tissues, or epibiotic, when it lives on the external surface of the host. In particular, epibiosis is a spatially close association between two or more organisms, the *epibiont* that colonizes the surface of live substrates, and the *basibiont* that gives support to the former (Wahl, 1989; Fernández-Leborans & Gabilondo, 2006). Interestingly, polychaetes have also been described as epibionts of a variety of sessile organisms, such as sponges (e.g., Pawlik, 1983; Lattig & Martin, 2009, 2011a, b; Lattig, Martin & Aguado, 2010; Álvarez-Campos, San Martín & Aguado, 2012), corals (e.g., Glasby & Watson, 2001; Martin *et al.*, 2002 Glasby *et al.*, 2012; Álvarez-Campos San Martín & Aguado, 2013), bryozoans (e.g., Morgado & Amaral, 1985), and cirripeds (Hernández, Muñoz & Rozbaczylo, 2001). Even though the majority of studies dealing with polychaetes associations, including the aforementioned, describe relationships in which the polychaete is the symbiont, they also play an important role as hosts of other organisms, such as other polychaetes, copepods, loxosomatids, nematods, and also protozoans (e.g. Nielsen, 1964; Martin & Britayev, 1998; Rangel *et al.*, 2009; Wakeman & Leander, 2013). In this study, we report for the first time the role of several species of syllid polychaetes as hosts of peritrichid ciliate protozoan epibionts.

Ciliate protozoans are commonly reported as internal parasites of numerous metazoans (Barel & Kramers, 1977; Taboada *et al.*, 2013) including polychaetes (Wakeman & Leander, 2013 and references herein). In addition, ciliates living on the external surfaces of a wide variety of aquatic Metazoa are commonly observed, most of

them belonging to the subclass Peritrichia (e.g., Corliss, 1961, 1979; Kahl, 1930–1935; Baldock, 1986; Smith, 1986; Weissman *et al.*, 1993; Gilbert & Schröder, 2003; Regali-Seleglim & Godinho, 2004; Fernández-Leborans, 2010; Sedlacek *et al.*, 2013). It is worth noting that, despite their ubiquitous presence as epibionts of aquatic metazoans, ciliate peritrich epibionts have only been reported in just a handful of polychaete species (Knox & Hicks, 1973; Magnini & Verni, 1988; Arias, Anadón & Paxton, 2010).

We expand here the knowledge on polychaete epibionts, reporting for the first time the occurrence of epibiont ciliate protozoans of the subclass Peritrichia from two different genera (*Rhabdostyla* and *Cothurnia*) on eleven different species of polychaetes within the family Syllidae: newly collected specimens of *Syllis magdalena* Wesenberg-Lund, 1962, *Syllis* sp.1, *Syllis* sp.2, *Salvatoria concinna* (Westheide, 1974) n. comb., *Salvatoria* sp., *Syllis prolifera* Krohn, 1852, *Syllis elongata* (Johnson, 1901), and museum specimens of *Typosyllis microoculata* (Hartmann-Schröder, 1965), *Typosyllis macropectinans* Hartmann-Schröder, 1982, *Parapionosyllis papillosa* (Pierantoni, 1903). Interestingly, we found two new species of epibionts of the genus *Rhabdostyla*, *Rhabdostyla mapuche* n. sp. and *Rhabdostyla taboadai* n. sp., and one new species of the genus *Cothurnia*, *Cothurnia kiwi* n. sp. (Table 1). Some species of *Rhabdostyla* have been previously reported as epibionts of other polychaete families, but noteworthy, the association between *Cothurnia* sp. and the phylum Annelida has never been observed before.

Material and Methods

Specimens of *Syllis magdalena*, *Salvatoria concinna* n. comb., *Salvatoria* sp. and *Syllis* spp. were collected in Central Chile (Las Cruces, Valparaíso Region; 33°30.06'S, 71°37.55'W) in January 2013, on intertidal algae including holdfasts of *Lessonia spicata*, and thalli of *Corallina* sp. and *Gelidium* sp. *Syllis elongata* specimens were collected in Peru (Albacora, Tumbes, Lima; 12°08.37'S, 77°01.36'O), by SCUBA on unidentified sponges at 20 m depth, in March 2013. Specimens of *Syllis prolifera* were collected by snorkeling and SCUBA in the Mediterranean Sea (Punta Santa Anna, Blanes, Spain; 41°40.25'N, 02°48.06'E), among an unidentified brown alga, in September 2013. Samples of *Prosphaerosyllis magnoculata* were collected by SCUBA diving in the East (Wellers Rock, Dunedin; 45°50.52'S, 170°37.31'E, 5 m

depth) and West (Tauranga Bay, Cape Foulwind; 41°46.23'S–171°27.12'E, intertidal) coasts of New Zealand, on unidentified algae during February 2012. *Typosyllis macropectinans*, was loaned from the Australian Museum (AM), originally collected in East Australia (North of Batemans Bay, New South Wales; 36°43.59'S, 149°59.16'E), in 2004. The samples of the species originally described as *Typollis microoculata* were loaned by the Hamburg Zoological Museum (HZM), originally collected in Hawaii (Maui; 20°45.09'N, 156°29.26'W). Type material of *Parapionosyllis papillosa* was not available, but original description and drawings from Pierantoni (1903) and (Fauvel, 1923) as well as specimens identified and described by Campoy (1982) were examined at the Museum of the Universidad de Navarra.

The newly collected samples were sorted using an Olympus SZ61 stereoscope, and fixed in either 10% formalin buffered in seawater, 96% ethanol or 2.5% glutaraldehyde solution. Syllids from Chile were studied under an Olympus CX21 microscope with an attached Micro-imaging digital camera at Estación Costera de Investigaciones Marinas de Las Cruces (ECIM). The rest of the specimens were studied under a Nikon Optiphot microscope with a differential interference contrast system (Nomarsky) at Universidad Autónoma de Madrid and a Zeiss Axioplan II compound microscope connected to a ProgRes C10 Plus digital camera (Jenoptics, Jena) at CEAB. For scanning electron microscopy (SEM), the specimens were critically point dried with an Emitech K850 Critical Point Dryer, gold-coated with a Q150T-S Turbo-Pumper Sputter Coater, and examined with a Hitachi S-3000N electron microscope at the Servicio Interdepartamental de Investigación (SIDI) of the Universidad Autónoma de Madrid (UAM) and a HITACHI HIGH-TECH TM3000 Tabletop at CEAB. For transmission electron microscopy (TEM), specimens fixed in 2.5% glutaraldehyde solution in 0.4M PBS with NaCl were postfixed in osmium tetroxide 1% in PBS, dehydrated in an ascending acetone series, and further embedded in epoxy resin and viewed in a JEOL 1010 microscope at the Serveis Científico-Técnicos at Universitat de Barcelona (UB). Protozoan epibionts were identified following Fernández-Leborans & Castro de Zaldumbide (1986); they were isolated and treated using the silver carbonate staining technique and also methyl green and neutral red staining. Light microscope images and morphometric data of the epibionts were obtained using Image Analysis (KS300 Zeiss). All newly collected specimens were deposited at the Museo Nacional de Ciencias Naturales de Madrid (MNCN).

Results

This is the first formal report of peritrich ciliate protozoans living as epibionts of syllid polychaetes. Numerous epibiont populations of *Rhabdostyla* spp. and *Cothurnia* sp. were observed living on newly collected and museum specimens of eleven different species of the family Syllidae. Interestingly, among the ciliate epibionts, two new species of *Rhabdostyla* and one new species of *Cothurnia* have been identified and described here (see taxonomy section below); *Rhabdostyla mapuche* n. sp. was observed on *S. magdalena*, *Syllis* sp.1, *Syllis* sp.2, *Salvatoria concinna* n. comb. and *Salvatoria* sp. from Chile. *Rhabdostyla taboadai* n. sp. was discovered on *S. prolifera* from the Spanish Mediterranean coast and *Cothurnia kiwi* was identified on *P. magnoculata* specimens from New Zealand (Figs. 1–5). Additionally, unidentified *Rhabdostyla* spp. epibionts were observed on *S. elongata* from Peru and *T. macropectinans* from Italy (Fig. 6). Two Syllidae museum specimens originally described as *T. microoculata* and *P. papillosa*, also presented ciliate epibionts of the genus *Rhabdostyla* that were misunderstood as papillae (Fig. 7). This overlooked epibiosis of ciliates on syllids might have important taxonomic implications, since the papillae have been considered as a valid character to distinguish among species or to erect new species. Indeed, *Typosyllis microoculata* is a synonymy of *S. prolifera* (see taxonomy section below).

It is also worth noting that this symbiotic relationship between protozoans and syllids is not restricted to a single geographical area, since the specimens presenting peritrichid ciliates described here have been observed in the Pacific Ocean (Chile, New Zealand, Australia, Hawaii) and Western Mediterranean Sea (Spain and Italy) (Table 1).

Association of syllids and ciliates

The epibiotic relationship between ciliates and polychaetes occurred in 22 specimens collected from holdfasts and thalli of several algae (Table 1). In every syllid specimen, ciliates were present in most segments at intersegmental furrows, close to parapodial bases, including the museum specimens originally described as *T. microoculata* and *P. papillosa* (Figs. 1–7). In addition, *Syllis magdalena* and *Syllis* sp. 2 presented ciliates on the prostomium and *S. elongata* around the nuchal organs, mouth

opening, and anterior cirri (Fig. 3G, 6A–B). In most cases, only one ciliate was found on the base of the parapodia in all specimens (Fig. 1, 3A–F, 4A, G, 5A–C, 6A–C). However, two or three ciliates may share the same parapodia, as observed in *Syllis* sp. 2 (Fig. 3H, 4C, 5D). Only in *T. macropectinans* most of the posterior ventral part was covered by a large number of ciliates (Fig. 6 D–F).

Taxonomic account of ciliate protozoans

Phylum Ciliophora Doflein, 1901

Subphylum Intramacronucleata Lynn, 1996

Class Oligohymenophorea De Puytorac *et al.*, 1974

Subclass Peritrichia Stein, 1859

Order Sessilida Kahl, 1933

Family Vaginicolidae de Fromentel, 1874

Genus *Cothurnia* Ehrenberg, 1831

Diagnosis. Loricated peritrichs with one or two zooids per lorica. Lorica attached aborally by stalk. Lorica without valves or operculum. Inner part of lorica may present internal lining or septum that can contain a mesostyle. Zooid may attach to lorica directly or via an endostyle (Warren & Paynter, 1991; Lynn & Small, 2000).

Ecology. Usually found as epibionts in many crustacean groups, including copepods, decapods, amphipods, isopods, and ostracods. Two species have been also found on tanaids (Fernández-Leborans & Sorbe, 2003). This is the first report of the genus living in epibiotic association with the phylum Annelida.

Cothurnia kiwi n. sp. Álvarez-Campos, Fernández-Leborans & San Martín

Figure 1, 2A

Material examined. New Zealand: Holotype (MNCN 39.01/5), 3 paratypes (one mounted for SEM) (MNCN 39.02/6-8), Tauranga Bay, Cape Foulwind, 41°46.23'S–171°27.12'E, unidentified coralline alga, intertidal, February 9th, 2012; Two paratypes (MNCN 39.02/9-10), Wellers Rock, Dunedin, 45°50.52'S, 170°37.31'E, unidentified

red alga, 5 m depth, February 7th, 2012. One paratype (MNCN 39.02/11), Akaroa Bay, 43°48.50'S–172°57.15'E, unidentified coralline alga, intertidal, February 5th, 2012.

Comparative material. See Table 2 for comparisons.

Description. Elongated pseudocylindrical lorica of 40.87–53.14 µm long and 16.81–19.2 µm wide; lorica shows transverse furrows and ridges from opening to rear end, with grooves more pronounced in middle area; opening of the lorica (10.5–13.7 µm) shorter than maximum body width with edges curved outward. Ciliate body 26.98–36.05 µm long, and 14.72–16.64 µm wide, connected to external stalk by a mesostyle; mesostyle presents longitudinal striation. External stalk 11.1–22.5 µm long and 2.5–2.08 µm wide. Oval macronucleus (7.36–11.09 µm long; 8.58–10.4 µm wide) located in anterior half of the body. Spherical micronucleus located near the macronucleus.

Remarks. *Cothurnia kiwi* n. sp. is characterized by having a mesostyle but lacking an endostyle, and by having an oval anterior macronucleus. *Cothurnia kiwi* n. sp. is compared to the most similar species of the genus in Table 2. *Cothurnia curvula* and *C. mobiusi* lack mesostyle and endostyle, and have a wider lorica than *C. kiwi* n. sp. *Cothurnia cyclopis* and *Cothurnia cytheridae* differ from *C. kiwi* n. sp. in having mesostyle and endostyle; the latter also presents a larger smooth lorica. *Cothurnia membranoloricata* has a shorter external stalk than *C. kiwi*.

Ecology. Attached to the base of parapodia in specimens of *Prosphaerosyllis magnoculata*.

Distribution. New Zealand

Etymology. The name refers to “kiwi”, a common term used for New Zealand people.

Family Epistylididae Kahl, 1933

Genus *Rhabdostyla* Kent, 1881

Diagnosis. Solitary peritrich ciliates with short non-contractile stalks, mostly inverted bell-shaped bodies and peristome with well-defined lip (Kent, 1880–81; Kahl, 1930–35; Lynn & Small, 2000). Taxonomical characters for identification of currently known species are summarized in Table 2.

Ecology. Mostly epibionts of freshwater invertebrates, particularly crustaceans, insects and oligochaetes (Kahl, 1935; Precht, 1935; Nenninger, 1948; Fernandez-Leborans & Tato-Porto, 2000; Regali-Selegim & Godinho, 2004). Some species also reported in

marine ecosystems, in association with different invertebrates, including polychaetes (Table 2). This is the first report of the genus in association with Syllidae.

Rhabdostyla mapuche n. sp. Álvarez-Campos, Fernández-Leborans & Verdes

Figures 2B, 3, 4A–E

Material examined. Chile, Valparaíso Region, Las Cruces, 33°30.06'S–71°37.55'W: Holotype (MNCN 39.02/12) and 7 paratypes (one mounted for SEM and one fixed for TEM) (MNCN 39.02/13–19), holdfast of *Lessonia spicata*, intertidal, February 14th, 2012; one paratype (MNCN 39.02/20), thallus of *Gelidium* sp., intertidal, February 14th, 2012; 4 paratypes (MNCN 39.02/21–24), thalli of *Corallina* sp. and *Gelidium* sp., intertidal, February 14th.

Comparative material. See Table 3 for comparisons.

Description. Body shape globular to spherical, 23–31 µm long, 16–19 µm wide; when contracting, posterior and anterior folds cover contracted part, appearing oval with a deep indentation that contains part of contracted posterior end. Peristomial (oral) disk 20–23 µm long, with 9–14 ciliary bands. Macronucleus c-shaped, anterior, near cell periphery. Cortex distinctly transverse-striated, with 36–39 silverlines. Micronucleus round, close to macronucleus. Stalk short, thick, 3–6 µm long, 4–6 µm wide, with longitudinal sinuation. Basal disk absent.

Remarks. *Rhabdostyla mapuche* n. sp. is unique among the genus because of its small size. It differs from *R. taboadai* n. sp. in its shorter stalk and lack of basal disk. Unlike *R. taboadai* n. sp., *R. variabilis*, *R. nereicola* and *R. arenaria*, *R. mapuche* n. sp. has a c-shaped macronucleus similar to *R. commensalis* but longitudinally located. *Rhabdostyla scyphoides* is similar in body shape but larger and not striated and also differs from *R. libera* in body shape and striation.

Ecology. Attached to intersegmental furrows, close to parapodial bases of *Syllis magdalena*, *Syllis* sp. 1 and *Syllis* sp. 2., *Salvatoria concinna* n. comb., and *Salvatoria* sp. Also attached to prostomium of *Syllis magdalena* and *Syllis* sp. 2.

Distribution. Central Chile.

Etymology. The name refers to the Mapuche, a group of indigenous inhabitants of south-central Chile and south-western Argentina.

Rhabdostyla taboadai n. sp. Álvarez-Campos, Fernández-Leborans, Riesgo & Martin

Figures 4E, 5

Material examined. Mediterranean Sea: holotype (MNCN 39.02/25) and 2 paratypes (one mounted for SEM) (MNCN 39.02/26-27), Punta Santa Anna, Blanes, Spain, 41°40.25'N, 02°48.06'E, unidentified brown alga, 3 m depth, September 23rd, 2013.

Comparative material. See Table 3 for comparisons.

Description. Body shape globular, elongated, 30–45 µm long, 15–23 µm wide, anterior end rounded to slightly projecting; when contracting, posterior and anterior folds cover the contracted part, appearing oval, with deep indentation containing part of contracted posterior end. Peristomial (oral) disk 26–29 µm long, with 11–18 ciliary bands.

Macronucleus multilobed, usually anterior, near cell periphery. Micronucleus absent.

Cortex distinctly transverse-striated, with 38–42 silverlines. Stalk 14–17 µm long, 7–9 µm wide, with longitudinal sinuation. Basal disk 8–12 µm in diameter.

Remarks. *Rhabdostyla taboadai* n. sp. is unique because of its oval and elongated body shape and its multilobular macronucleus. The rest of distinctive characters are discussed in *R. mapuche* remarks.

Ecology. Attached to intersegmental furrows, close to parapodial bases of *Syllis prolifera*.

Distribution. Costa Brava (Spanish North-Eastern Mediterranean coast).

Etymology. Named after Dr. Sergi Taboada, polychaetologist, colleague and friend, for his help collecting and sorting the Mediterranean material and for his useful advice and support.

Taxonomic account of syllids

Salvatoria concinna n. comb. (Westheide, 1974)

Brania concinna Westheide, 1974, 44: 91, fig. 41

San Martín (2005) rescued *Salvatoria* McIntosh, 1885 for some species previously described as *Brania* Quatrefages, 1866. The genus formerly included *Brania concinna* Westheide, 1974 from Galápagos Islands, but the species is transferred here to the genus *Salvatoria* because it broods dorsally. This is also the first report of *Salvatoria concinna* (Westheide, 1974) n. comb. for Chilean waters.

Syllis prolifera Krohn, 1852

Typosyllis microoculata Hartmann-Schröder, 1965, 18(1): 66–76. Licher (1999): 133, fig. 60.

Syllis prolifera San Martín (2003): 344, figs. 186, 187.

Specimens of *Typosyllis microoculata* described by Hartmann-Schröder (1965) from Hawaii (Paratypes, HZM P-14498) were examined and their characteristic papillae were found to be in fact misinterpreted ciliate protozoans (Fig. 7A). Without these “papillae” *Typosyllis microoculata* is identical to the Mediterranean *Syllis prolifera*, and thus considered a synonymy.

As suggested by previous authors (San Martín, 2003; Gil, 2011) a similar situation might be occurring with *Parapionosyllis papillosa*, which was described bearing papillae on intersegmental furrows, with a remarkable similarity to ciliate epibionts described herein (Fig. 7B). However, we were not able to study the type material and therefore, we cannot be sure about the taxonomical status of this species.

Discussion

This is the first study formally reporting the presence of peritrich ciliate protozoans living as epibionts of several polychaete species of the family Syllidae. Despite previous studies already suggested that the diagnostic character “papillae” used to describe some species, could be parasitic ciliates (San Martín, 2003; Gil, 2011) this epibiotic association was not previously confirmed. The occurrence of peritrich ciliates as epibionts of invertebrates is ubiquitous in marine ecosystems but, surprisingly, there are just a handful of studies describing epibiotic associations between the aforementioned protozoans and polychaete annelids. Knox & Hicks (1973) found numerous specimens of *Vorticella* spp. on the dorsal surface of the anterior end of the body of the onuphid *Brevibrachium maculatum* (Estcourt, 1966) (as *Rhamphobranchium maculatum*) from New Zealand. Magagnini & Verni (1988) discovered epibiotic peritrich ciliates of the genus *Scyphidia* living in association with the archiannelid *Nerilla antennata* Schdmit, 1848 from Italy, and more recently Arias *et al.* (2010) reported the presence of *Epystilis* sp. on the gills and first parapodia of the onuphid *Diopatra marocensis* Paxton, Fadlaoui & Lechapt, 1995 from northern Spain. Several *Rhabdostyla* species have also been described as epibionts of polychaetes from

different families (see Table 3). Only Magagnini & Verni (1988) pointed out the possible biological and ecological implications for both partners, discarding parasitism as no external damage was observed in the host, even when ciliates were present in large numbers. Accordingly, they defined the association as a classical example of ectocommensalism (*sensu* Puytorac *et al.*, 1987).

Ciliate associations have been traditionally reported in crustaceans, where detrimental effects on host linked to the presence of epizoid protozoa seemed to be the rule. For instance, they may compete for food or cause negative effects on locomotion or sensory functions turning hosts either more vulnerable to predation or less competitive with non-infected crustaceans (Herman & Mihursky, 1964; Herman *et al.*, 1971; Evans *et al.*, 1981; Nagasawa, 1986; Scott & Thune, 1986; Kankaala & Eloanta, 1987; Nagasawa, 1988). In some cases, even the basibionts life is threatened due to the asphyxiation caused by occlusion of functional respiratory surface by the physical presence of large numbers of protozoans on the gills (Lightner, 1975; Fisher, 1977; Couch, 1978). However, the presence of peritrich ciliates can also benefit their host, since some of them contain toxins that may act as defenses against predation (Henebry & Ridgeway, 1979). In turn, all authors agree that increased food availability is the major advantage obtained by the epibionts from being associated to a motile substratum. It is not the focus of this study to evaluate the potential benefits or disadvantages that this epibiosis might provide to syllids, however, no impairment of swimming efficiency, or other external harm, was noticed in the specimens carrying ciliate protozoans studied herein. To this end, future studies would be certainly required.

The fact that the epibiotic association between syllids and ciliates has been so rarely observed and reported in a group that has been extensively studied, particularly during the last 30 years, is certainly intriguing. Many authors have largely discussed on the ecological importance of protozoans in aquatic ecosystems and their possible use as bioindicators, because they usually thrive in oxygen poor waters (e.g., Spoon, 1965; Antipa, 1977; Fisher, 1977; Scott & Thune, 1986). Global warming and other climatological events might lead to lowered oxygen content of the world oceans (Keeling & García, 2002), and therefore the anthropogenically induced hypoxia in the seawater may have strong consequences on marine symbiotic associations. Interestingly, all our newly collected samples were obtained from harbors or gathered in the vicinity of anthropogenically-disturbed zones, and therefore the induced hypoxia

may be suggested as one of the causes explaining the presence of ciliated peritrich protozoans in our syllid specimens, although no measurements of oxygen content have been obtained yet. However, *P. papillosa* was described by Pierantoni (1903) in Northern Italy and *T. microoculata* by Hartmann-Schroder (1965) in Hawaii, and at those times they were also carrying similar ciliate protozoans. We ignore if those specimens were collected from reportedly organically enriched areas, and therefore, alternative explanations for the presence of symbiotic protozoans might either be a rising number of epibionts per host (for still unknown biological or ecological reasons) or, more likely, a recent increased dedication of the observers. Additionally, the effects of global warming on the protozoans ecology might generate a shift on hosts that could explain their more frequent detection on syllids (Vickerman, 1992).

Regardless of the causes of this newly reported epibiosis, there is a remarkable taxonomic implication affecting the species descriptions within the family Syllidae. Given the cases of *P. papillosa* and *T. microoculata*, we might expect an overestimation of the number of syllid species. There might be other cases where the presence of “papillae” (i.e. misinterpreted protozoans) may have been a key character leading to distinguish one “species” from another, especially when preserved specimens are observed (which is a common practice in taxonomic studies). The fixation method used to preserve polychaetes leads to the contraction of the ciliate epibionts, which coupled with their small size poses serious difficulties to differentiate them from simple papillae. Furthermore, this argument may certainly be extensive to other polychaete families (e.g. Sphaerodoriidae), and therefore we strongly suggest assessing the real origin of papillae-like structures in polychaetes in routine identification protocols.

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Figure legends

Figure 1. *Cothurnia kiwi* n. sp. on *Prosphaerosyllis magnoculata* (MNCN 39.02/8). A) Scanning electron micrograph of the anterior part of *P. magnoculata* showing several individuals of *C. kiwi* n. sp. (arrows). B-C) Close-up micrographs of the

epibiont *C. kiwi* n. sp. on the intersegmental furrows of the annelid. D) Light microscopy picture of the stained protozoan showing the lorica (l) and the external stalk (es).

Figure 2. Line drawings of the new species of ciliate protozoans. A) *Cothurnia kiwi* n. sp. (MNCN 39.01/5). B) *Rhabdostyla mapuche* n. sp. (MNCN MNCN 39.02/12). C) *Rhabdostyla taboadai* n. sp. (MNCN 39.02/25) Abbreviations: l, lorica; ma, macronucleus; mi, micronucleus; ms, mesostyle; es, external stalk; pd, peristomial disk; s, stalk; bd, basal disk.

Figure 3. Light microscopy pictures of *Rhabdostyla mapuche* n. sp. on different Syllidae species. A) Anterior part of *Salvatoria* sp. (MNCN 39.02/21) showing several individuals of *R. mapuche* n. sp. (arrows). B) Close-up picture of the epibiont on the intersegmental furrows of the syllid. C) Stained protozoan on *Syllis* sp. 1 (MNCN 39.02/16) showing the macronucleus (ma) and micronucleus (mi). D) Anterior part of *Salvatoria concinna* n. comb. (MNCN 39.02/24) showing several individuals of the epibiont (arrows). E) Close-up picture of the epibiont on the intersegmental furrows of the syllid. F) Close-up picture of epibiont showing the stalk (s) and vacuoles (v). G) Anterior part of *Syllis* sp. 2 (MNCN 39.02/20) showing several individuals of the epibiont (arrows). H) Close-up micrograph of the epibiont on the intersegmental furrows of the syllid. I) Close-up micrograph of epibiont showing the lorica (l), vacuoles, the micronucleus, macronucleus and the stalk.

Figure 4. *Rhabdostyla* n. spp. on different Syllidae species. A) Scanning electron micrographs of the anterior end in ventral view of *Syllis* sp. 2 (MNCN 39.02/20) showing one epibiont in the base of tentacular cirri. B) Anterior and midbody segments, dorsal view, of *Syllis* sp. 2 showing several ciliates (arrows). C) Close-up micrograph of the epibiont on the intersegmental furrows of *Syllis* sp. 2. D) Close-up micrograph, lateral view, of two ciliates showing the stalk (arrows). E) Close-up micrograph of an epibiont, apical view. F) Transmission electron micrograph of *R. mapuche* n. sp. on *Syllis* sp. 1 (MNCN 39.02/16). Note the arrow pointing to the basal part of the epibiont. G) Scanning electron micrograph of *Rhabdostyla taboadai* n. sp. on *Syllis prolifera* (MNCN 39.02/26), showing the stalk (arrow).

Figure 5. Light microscopy pictures of *Rhabdostyla taboadai* n. sp. on *Syllis prolifera* (MNCN 39.02/25) A) Anterior part of the syllid showing an individual of *R. taboadai* n. sp. (arrow). B-D) Close-up pictures of the epibiont on the intersegmental furrows of the syllid showing the stalk (arrow) and the peristomial disk (pd).

Figure 6. Scanning electron micrographs of unidentified *Rhabdostyla* species on two Syllidae species. A) Anterior end of *Syllis elongata* (MNCN 39.02/15296) showing several individuals of *Rhabdostyla* sp. 1 (arrows). B–C) Close-up micrographs of the epibiont on intersegmental furrows and tentacular cirri of *S. elongata*, showing the stalk (arrow). D) Posterior ventral part of *Typosyllis macropectinans* (AM W.41626) showing several individuals of *Rhabdostyla* sp. 2 (arrows). E–F) Close-up micrographs of the ciliates showing the stalk (arrow).

Figure 7. A) Light microscopy picture of *Syllis prolifera* (originally described as *Typosyllis microoculata*) from Hawaii (HZM.P.14498) showing the epibiotic ciliates on the intersegmental furrows (arrows). B) Line drawing (modified from Pierantoni, 1903) of *Parapionosyllis papillosa* from Italy, showing the epibiotic ciliates on the intersegmental furrows (arrows).

Table 1. Epibiotic ciliate protozoans identified in Syllidae polychaetes.

Table 2. Comparison between *Cothurnia kiwi* n. sp. and the most similar species of the genus.

Table 3. Comparison between *Rhabdostyla* new species and the most similar species of the genus.

A **B** **C**

EPIBIONTS	BASIBIONT	LOCALITY	COORDINATES	COLLECTION DATE	CATALOGUE NUMBER
Newly collected specimens					
<i>Cothurnia kiwi</i> <i>n. sp.</i>	<i>Prosphaerosyllis magnooculata</i>	Wellers Rock, Dunedin, New Zealand	45°50.52'S, 170°37.31'E	Feb 7 th , 2012	Holotype MNCN 39.01/5 2 Paratypes MNCN 39.02/6-7 1 Paratype SEM MNCN 39.02/8
		Tauranga Bay, Cape Foulwind, New Zealand	41°46.23'S, 171°27.12'E	Feb 9 th , 2012	2 Paratypes MNCN 39.02/9-10
		Akaroa Bay, New Zealand	43°48.50'S, 172°57.15'E	Feb 5 th , 2012	1 Paratype MNCN 39.02/11
<i>Rhabdostyla mapuche</i> <i>n. sp.</i>	<i>Typosyllis magdalena</i>	Las Cruces, Valparaíso Region, Chile	33°30.06'S, 71°37.55'W	Jan 14 th , 2013	Holotype MNCN 39.02/12 3 Paratypes MNCN 39.02/13-15
	<i>Syllis sp.1</i>	Las Cruces, Valparaíso Region, Chile	33°30.06'S, 71°37.55'W	Jan14 th , 2013	1 Paratype MNCN 39.02/16
	<i>Syllis sp.2</i>	Las Cruces, Valparaíso Region, Chile	33°30.06'S, 71°37.55'W	Jan 14 th , 2013	3 Paratypes MNCN 39.02/17-19
		Las Cruces, Valparaíso Region, Chile	33°30.06'S, 71°37.55'W	Jan 14 th , 2013	1 Paratype MNCN 39.02/20
	<i>Salvatoria sp.</i>	Las Cruces, Valparaíso Region, Chile	33°30.06'S, 71°37.55'W	Jan14 th , 2013	1 Paratype MNCN 39.02/21
	<i>Salvatoria concinna</i>	Las Cruces, Valparaíso Region, Chile	33°30.06'S, 71°37.55'W	Jan 14 th , 2013	3 Paratypes MNCN 39.02/22-24
<i>Rhabdostyla taboadai</i> <i>n. sp.</i>	<i>Syllis prolifera</i>	Punta Santa Anna, Blanes, Spain	41°40.25'N, 02°48.06'E	Sep 23 rd , 2013	Holotype MNCN 39.02/25 1 Paratype MNCN 39.02/26 1 Paratype SEM 39.02/27
<i>Rhabdostyla sp.1</i>	<i>Syllis elongata</i>	Albacora, Tumbes, Peru	12°08.37'S, 77°01.36'W	Mar, 2013	3 specimens SEM MNCN 16.01/15296
Revised museum specimens					
<i>Rhabdostyla sp.</i>	<i>Typosyllis macropectinans</i>	North of Batemans, New South Wales, Australia	36°43.59'S, 149°59.16'E	Mar 16 th , 2004	AM.W41626
<i>Rhabdostyla sp.</i>	<i>Typosyllis microoculata</i>	Maui, Hawaii	20°45.09'N, 156°29.26'W	May 19 th , 1959	1 Paratype HZM.P-14498

*Catalogue numbers for newly collected specimens correspond to ciliate epibionts. Catalogue numbers for revised museum specimens represent Syllidae species.

	<i>Cothurnia curvula</i>	<i>Cothurnia mobiusi</i>	<i>Cothurnia cyclopis</i>	<i>Cothurnia cytheridae</i>	<i>Cothurnia membranoloricata</i>	<i>Cothurnia kiwi n. sp.</i>
Lorica length (µm)	60	50	60	70	40-45	40.9-53.1
Lorica width (µm)	30	30	25	35	18-23	16.8-19.2
Lorica striation	central annular	smooth	furrows and ridges	smooth	smooth	furrows and ridges center thicker
Lorica aperture	15	20	?	30	20	10.5-13.7
Body length (µm)	60	70	80	45	55-57	26-36
Body width (µm)	8-10	15	20	20	12-15	14.7-16.6
Mesostyle	-	-	+	+	-	+
Endostyle	-	-	+	+	+	-
External stalk length (µm)	short, bulbous in attachment lorica	short	15	35	8-12	11.1-22.5
Macronucleus shape	straight, longitudinal	c-shaped, transversal	?	curved, longitudinal	?	oval, anterior
Basibiont	Harpacticoida	?	Harpacticoida	<i>Cytheridea</i> sp.	<i>Cladophora</i> sp.	<i>Prosphaerosyllis magnoculata</i>
Distribution	Tyrrhenian Sea	North Sea	?	?	Adriatic Sea	New Zealand
References	Entz, 1884	Stiller, 1939	Kahl, 1933	Kahl, 1933	Stiller, 1968	

	<i>Rhabdostyla libera</i>	<i>Rhabdostyla commensalis</i>	<i>Rhabdostyla arenaria</i>	<i>Rhabdostyla nereicola</i>	<i>Rhabdostyla scyphoides</i>	<i>Rhabdostyla variabilis</i>	<i>Rhabdostyla mapuche</i> n. sp.	<i>Rhabdostyla taboadai</i> n. sp.
Body shape	slight	barrel-shaped	?	?	cylindrical	?	oval spheroid	oval elongated
Length (µm)	?	?	56	55-62	?	60-90	23-31	30-45
Width (µm)	?	?	?	?	?	?	16-19	15-23
Oral disk shape and length (µm)	?	papillary projection	?	sheet-like	broad, not elevated	?	broad, convex elevated (20-23)	broad, convex elevated (26-29)
Ma shape	?	sausage-shaped, short central	ribbon-shaped, very long	?	?	kidney-shaped	c-shaped, longitudinal	multilobed
Body striation	thin, longitudinal	?	?	?	pellicle, smooth	?	transversal	transversal
Stalk striation		?	?	?	?	?	longitudinal	longitudinal
Stalk length (µm)	short	?	10	?	?	?	3-6	14-17
Stalk width (µm)	?	?	?	?	?	?	4-6	7-9
Basal disk length (µm)	?	?	?	?	?	?	-	8-12
Basibiont	Algae and aquaria walls	<i>Capitella capitata</i> , <i>Terebellides ströemii</i>	<i>Synapta inhaerens</i> , <i>Asterias rubens</i> , <i>Leptosynapta</i> sp., <i>Astropecten</i> sp.	<i>Nereis dimerii</i>	<i>Penaeus orientalis</i>	<i>Scoloplos armiger</i> , <i>Phyllodoce laminosa</i>	<i>Syllis magdalena</i> , <i>Syllis</i> sp.1, <i>Syllis</i> sp.2., <i>Salvatoria concinna</i> , <i>Salvatoria</i> sp.	<i>Syllis prolifera</i>
Distribution	?	?	Baltic Sea, Germany	Baltic Sea, Germany	?	Baltic Sea, Germany	Pacific Ocean, Central Chile	North-Eastern Mediterranean Sea, Spain
References	Kahl, 1933	Möbius, 1888	Cuenot, 1891	Precht, 1935	Song, 1986	Dons, 1918		